Bridges over Multiple Biotech Corridors

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Background

T-cell engager mechanism-of-action

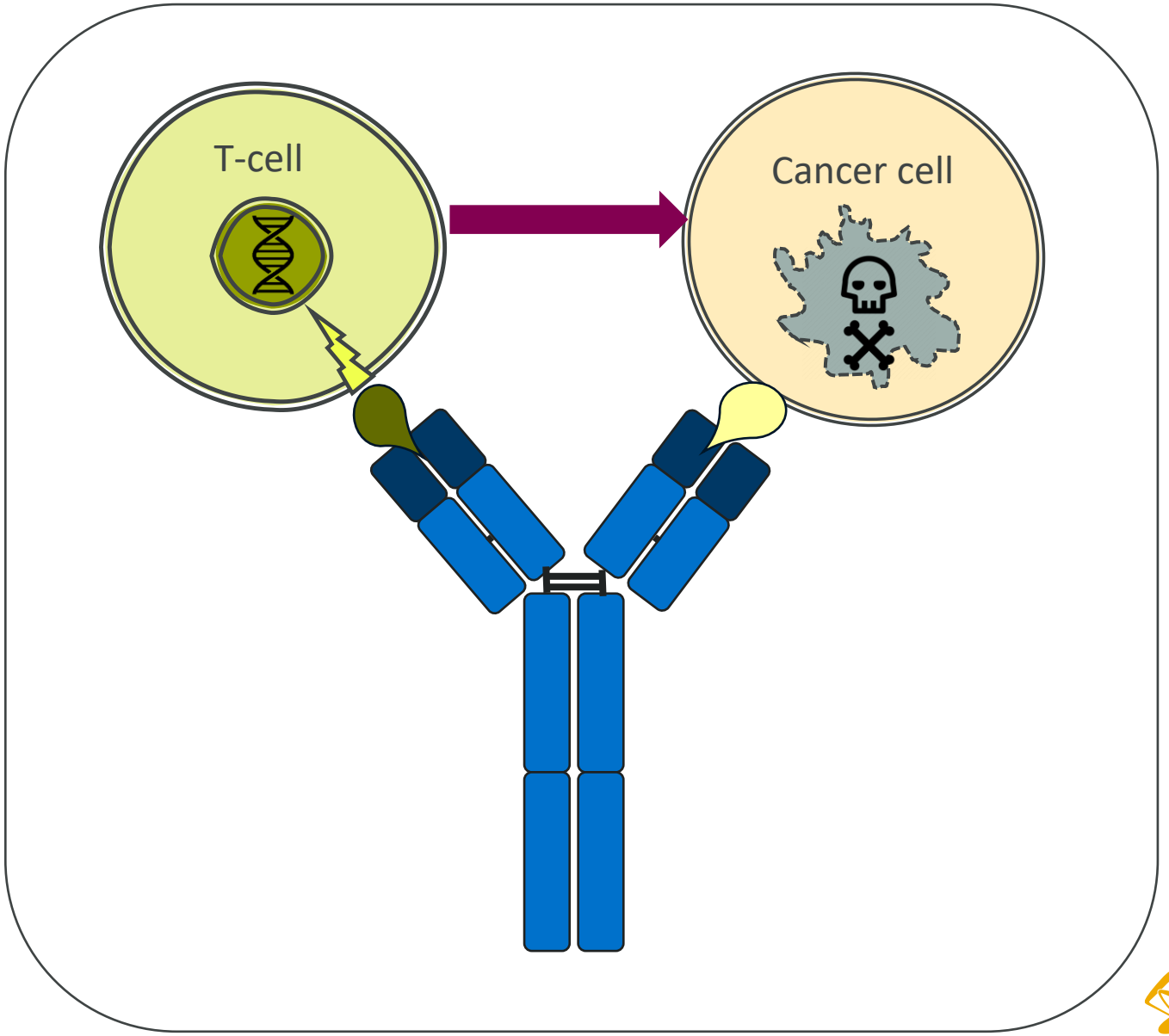
Acquired from startup and a partnered CMO

Manufacturing and analytical testing remained at CMO

MoA: T-cell Engager

- Simultaneously binds T-cells and cancer cells expressing a target antigen
- Activates T-cells to kill cancer cells

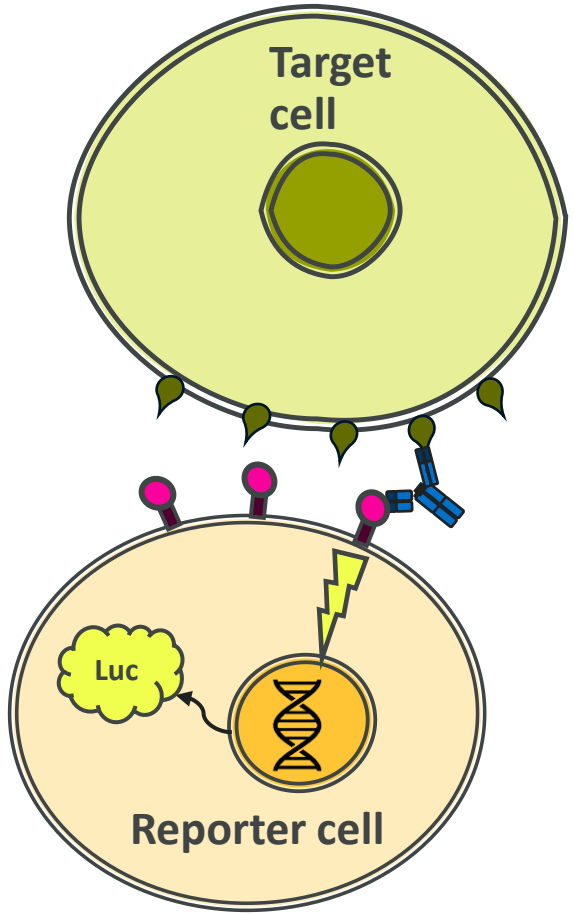
Fc effector function null



3 potency assays on lot-release and stability spec when acquired

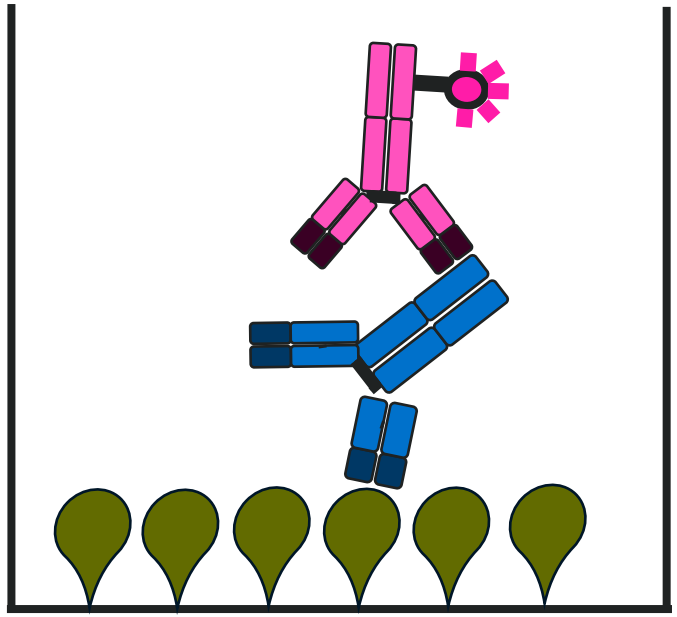
#1: Dual-cell Reporter Gene Assay (RGA)

- Reporter cell line (TCR expression)
- Target cell line (target antigen expression)



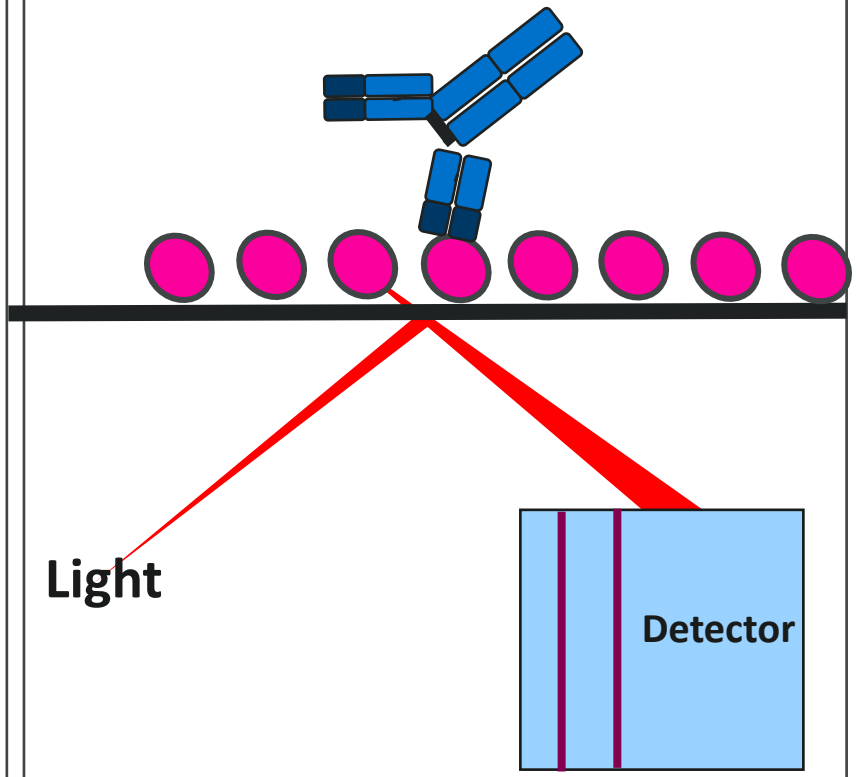
#2: Antigen-binding ELISA

- Purified target antigen protein
- HRP-conjugated anti-human Fc mAb



#3: TCR SPR Assay

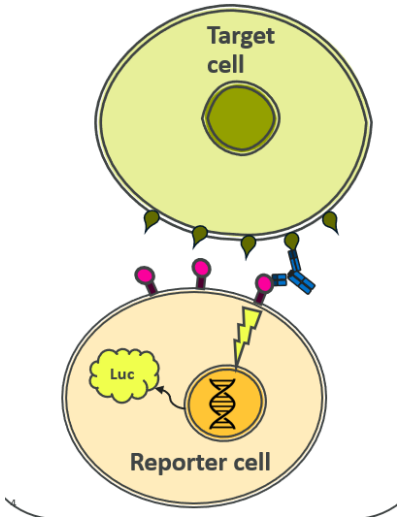
- Purified TCR-associated protein
- HRP-conjugated anti-human Fc mAb



Future strategy: Only RGA method on specification for potency

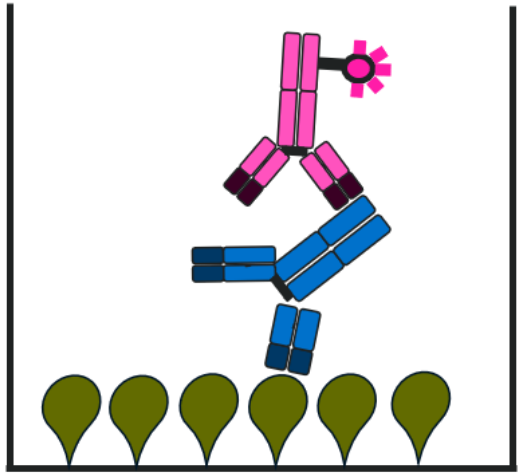
Dual-target RGA

Lot-release



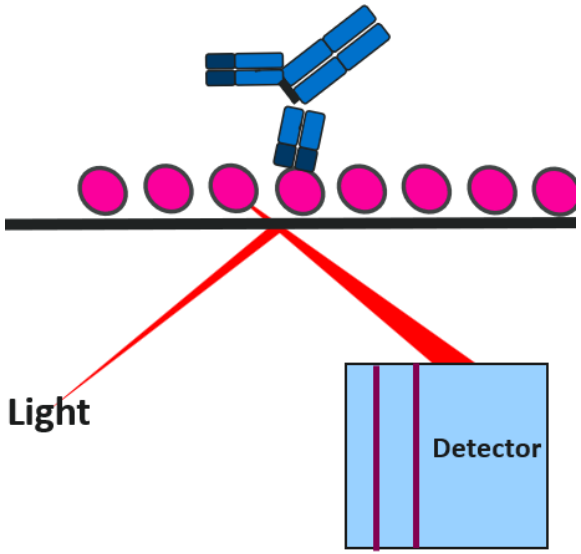
Antigen-binding ELISA

Characterization



TCR SPR

Characterization



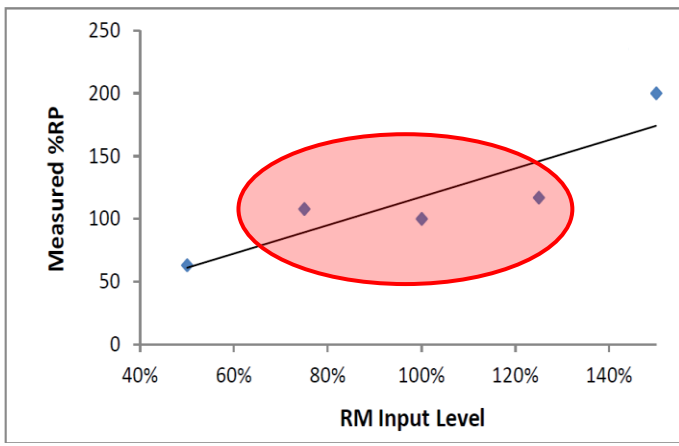
How:

1. Defend that RGA is cell-based and fully MoA-reflective. Binding assays are non-cell based and partially MoA-reflective.
2. Use ***method bridging*** to demonstrate RGA and binding assays are equivalent in method performance

Multiple challenges to address before and during bridging study

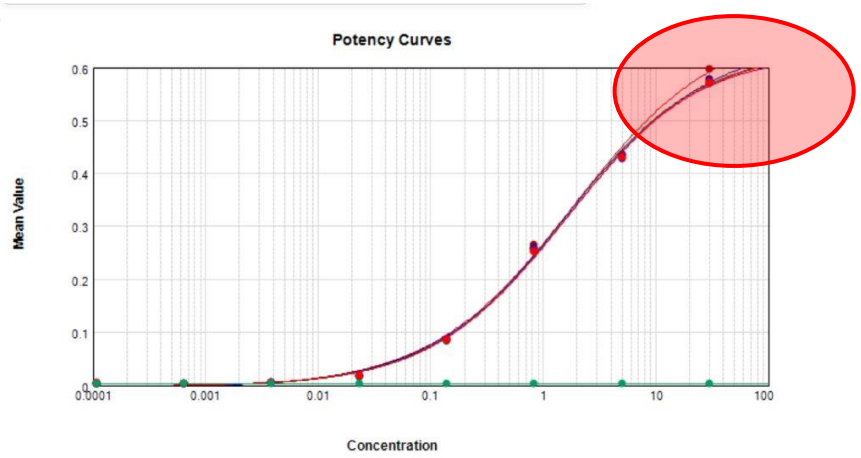
1. RGA performance not suitable for late-stage/commercial
2. Antigen-binding ELISA recurring system suitability failures
3. Simultaneous parallel process/formulation changes and CQA evaluations
4. Assays located at 2 different CMO sites

RGA Accuracy and Linearity



[A]	[B]
RP Input Level	Measured %RP
175%	369
150%	200
125%	117
100%	100
75%	108
50%	63
25%	47

Antigen-binding ELISA 4PL Curve



Blueprint to new potency assay strategy

AZ Gaithersburg, MD

Dual-target RGA

Re-develop

Dual-target RGA

Antigen ELISA

Optimize

Antigen ELISA

Transfer

Implement

Implement

CMO Site #1

Antigen ELISA (Lot-release)

Antigen ELISA

Dual-target RGA

Qualify

Dual-target RGA

Lot-release

Dual-target RGA

Qualify

Antigen ELISA

Characterization

Antigen ELISA

TCR SPR (Lot-release)

Verify

TCR SPR

Bridge

TCR SPR

Implement

Dual-target RGA

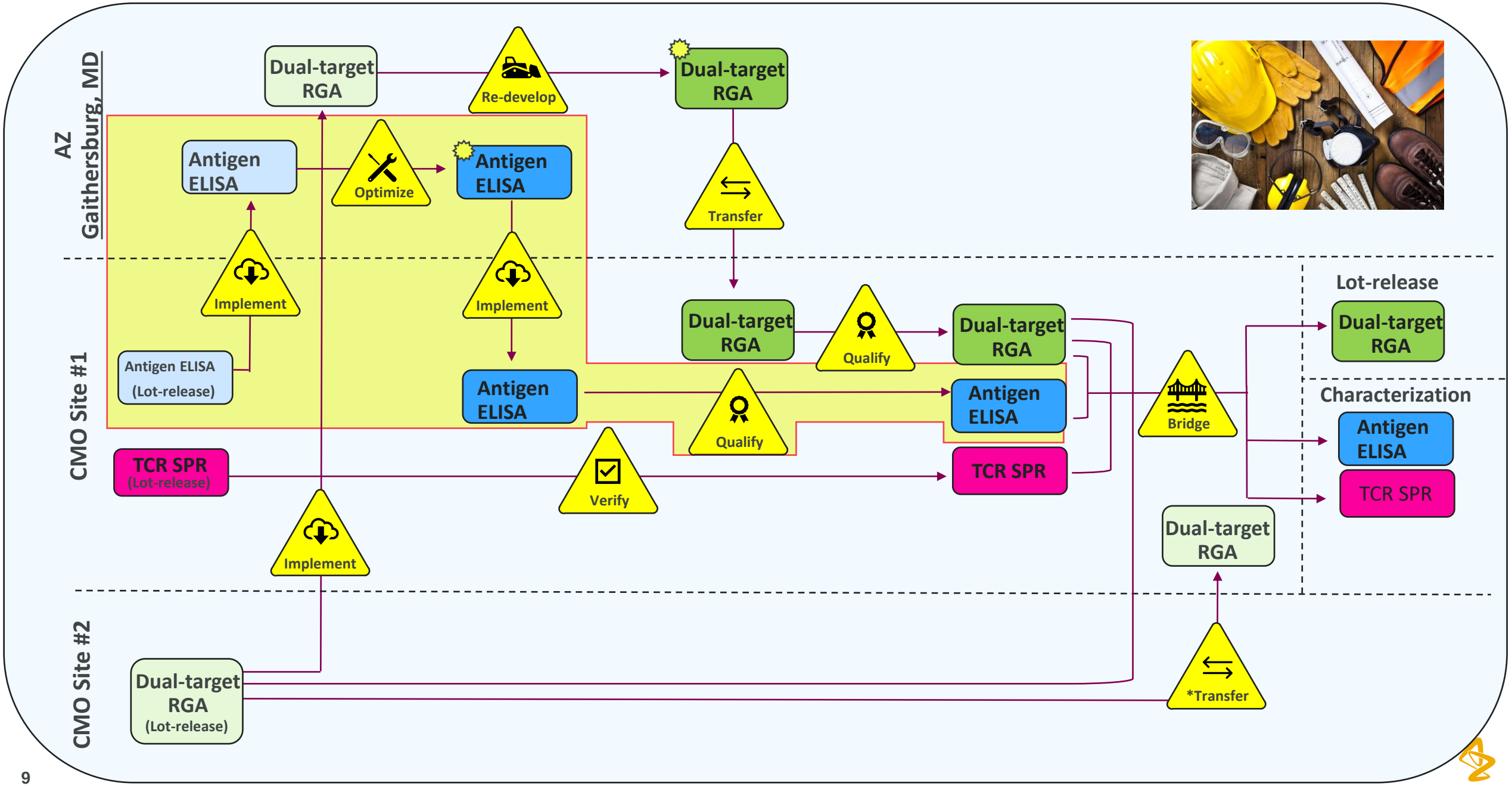
CMO Site #2

Dual-target RGA (Lot-release)

*Transfer

Antigen-binding ELISA Optimization

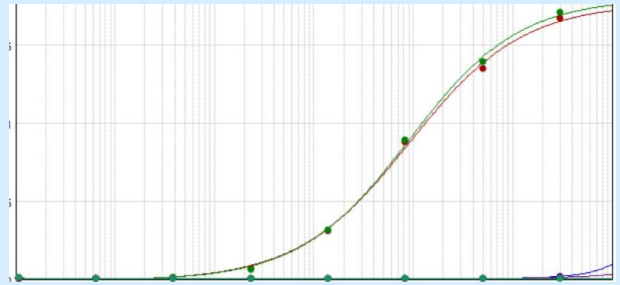
Optimizing and re-qualifying Antigen-binding ELISA



Antigen-binding ELISA optimization and re-qualification

#1: Implement method at AZ

- Confirmed ill-defined upper-asymptote across three potency levels and 3 independent runs.



#2: AZ identify assay issues

- Blocking buffer, assay buffer, and TMB reaction time all contributed to curve issues.

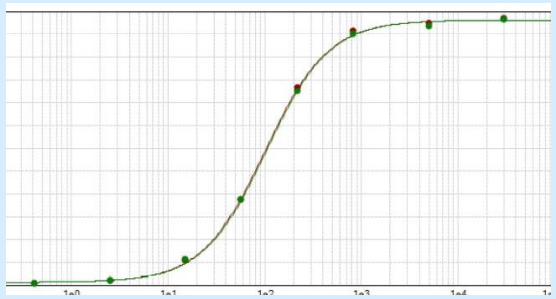
#3: AZ optimize assay

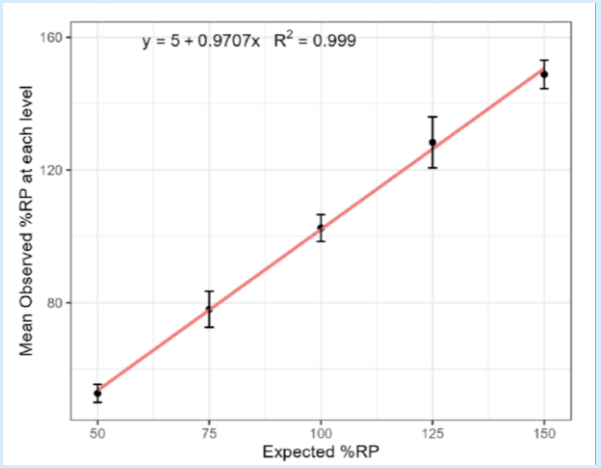
- Replaced buffers, increased incubation times, changed dilution scheme

#4: Implement at CMO

- CMO updated SOP and performed runs to confirmed improved performance

#5: CMO Requalified method





Parameter	Analysis	Result
Linearity	R^2 from linear regression	Pass
Accuracy	90% CIs[1] of mean accuracies across potency range	Pass
Repeatability	%CV of potency results[2]	Pass
Intermediate Precision	95% CI from VCA[3] model fitted to run-to-run variability	Pass
Range	Linearity, accuracy, and precision criteria	Pass
Specificity	Curve visualization	Pass
Stability-indicating	Potency trend across stability timepoints	Pass

[1]CI = confidence interval

[2]Potency results were generated from independent sample replicates prepared at the nominal assay starting concentration and tested on the same day

[3] VCA = variance component analysis

RGA Re-development

Optimizing and re-qualifying RGA

AZ
Gaithersburg, MD

Dual-target RGA
Re-develop
Dual-target RGA
Antigen ELISA
Optimize
Antigen ELISA
Transfer
Implement
Implement
Dual-target RGA
Qualify
Dual-target RGA
Lot-release
Dual-target RGA
CMO Site #1
Antigen ELISA (Lot-release)
Antigen ELISA
Qualify
Antigen ELISA
Characterization
Bridge
Antigen ELISA
TCR SPR (Lot-release)
Verify
TCR SPR
TCR SPR
Implement
Dual-target RGA
CMO Site #2
Dual-target RGA (Lot-release)
*Transfer

RGA re-development, transfer, and qualification

#1: Implement method at AZ

- 3 independent runs according to current SOP across 3 potency levels.

#2: Re-developed assay (use of DoE key to efficient redevelopment)

- Updated plate layout
- Optimized cell densities/ ratios and assay incubation time
- Changed luminescence reagent

#3: Transferred re-developed assay

Study Design

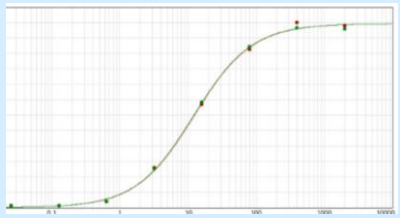
- One analyst at AZ performing 6 runs across 3 potency levels
- Two analysts at CMO performing 4 runs each across same 3 potency levels.
- Mean difference in accuracy and CIs calculated across potency levels and between sites.

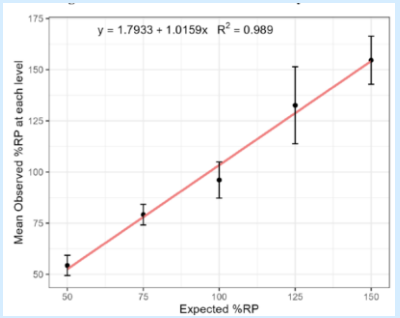
Criteria

1. 90% CI of mean accuracy difference between sites and at each potency level must be within specified upper/lower bounds.
2. 90% CI of mean overall accuracy difference between sites must be within specified upper/lower bounds.
3. Report precision

Potency level	CI lower bound result	CI upper bound result
Level #1 (50% RP)	Pass	Pass
Level #2 (100% RP)	Pass	Pass
Level #3 (150% RP)	Pass	Pass
Overall	Pass	Pass

#4: CMO qualified assay





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Specificity	Curve visualization	Pass
Stability-indicating	Potency trend across stability timepoints	Pass

Bridge Study Planning

Optimizing and re-qualifying Antigen-binding ELISA

AZ Gaithersburg, MD

Dual-target RGA

Re-develop

Dual-target RGA

Antigen ELISA

Optimize

Antigen ELISA

Transfer

Implement

Implement

CMO Site #1

Antigen ELISA (Lot-release)

Dual-target RGA

Qualify

Lot-release

Dual-target RGA

Antigen ELISA

Qualify

Characterization

Antigen ELISA

TCR SPR (Lot-release)

Verify

Dual-target RGA

Antigen ELISA

TCR SPR

Bridge

TCR SPR

Implement

Dual-target RGA

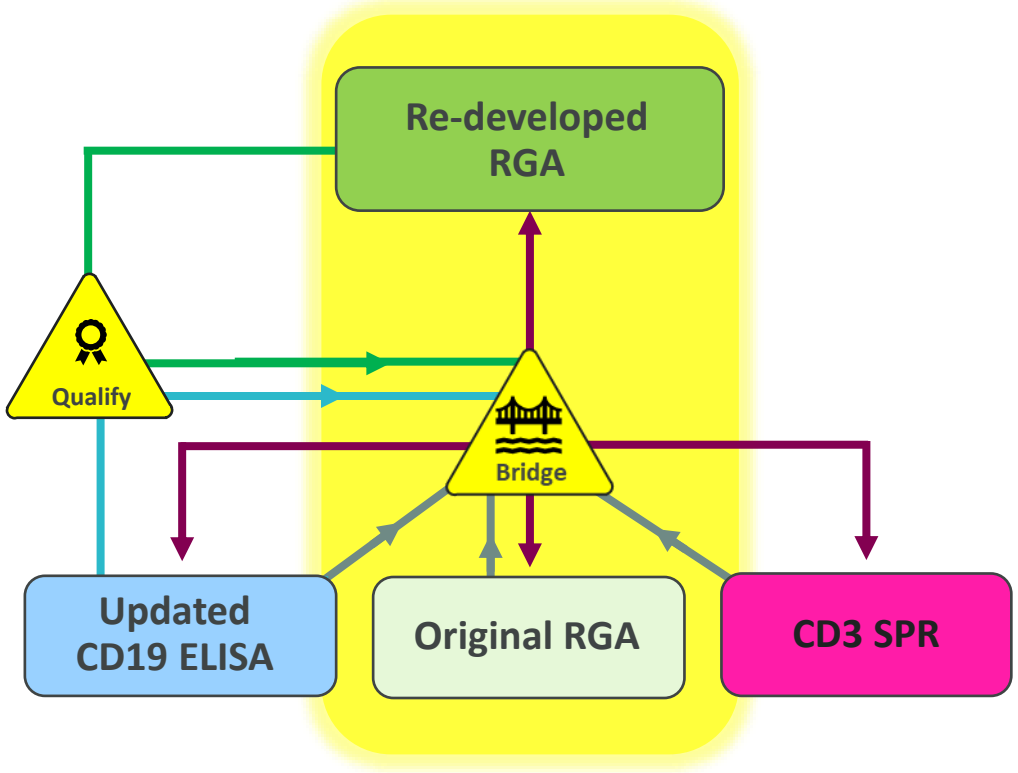
CMO Site #2

Dual-target RGA (Lot-release)

*Transfer

Bridge Design

Included language in protocol that any non-equivalency will be further investigated



Study Design and Criteria: Re-developed RGA vs each Assay

Purpose	Samples	Criteria
*Equivalency of Accuracy	• 50% RP (n=6) • 100% RP (n=6) • 150% RP (n=6)	90% CI of mean difference is within specified bounds at each potency level and overall.
Equivalency of lot-release testing	All DS and DP lots to date (n=2 per sample)	90% CI of mean difference is within specified bounds.
Stability-indicating	Forced degradation timepoints + non-degraded control	Demonstrate similar trends
Precision	Data from accuracy testing	Report Results

***6 independent runs distributed equally across 2 different analysts for each assay**

Original versus re-developed RGA at most risk of being non-equivalent

- Original assay demonstrated poor linearity and precision
- Want to accept non-equivalency if caused by superiority of the re-developed RGA. This is why we re-developed!

Bridge Results

Re-developed RGA versus Binding Assays: Equivalency of Accuracy

Equivalency Results of New RGA vs Antigen-Binding ELISA

Level	Mean Accuracy		Mean Difference	90% LCL	90% UCL	Outcome
	New RGA	ELISA				
50%	108.7	105.3	3.4	**-5.2**	**12.0**	**Pass**
100%	96.1	102.5	-6.4	**-13.9**	**1.1**	**Pass**
150%	103.1	99.2	3.9	**-2.7**	**10.4**	**Pass**
Overall	102.6	102.4	0.2	**-4.1**	**4.7**	**Pass**

Equivalency Results of New RGA vs TCR SPR

Level	Mean Accuracy		Mean Difference	90% LCL	90% UCL	Outcome
	New RGA	SPR				
50%	108.7	92.3	16.4	**8.2**	**24.6**	**Pass**
100%	96.1	93.7	2.4	**-4.9**	**9.7**	**Pass**
150%	103.1	95.7	7.4	**0.9**	**13.9**	**Pass**
Overall	102.6	93.9	8.7	**4.6**	**12.9**	**Pass**

Equivalency Results of New RGA vs Old RGA

Level	Mean Accuracy		Mean Difference	90% LCL	90% UCL	Outcome
	New RGA	Old RGA				
50%	108.7	96.0	12.7	**4.1**	**21.4**	**Pass**
100%	96.1	116.0	-19.9	**-82.5**	**42.7**	**Fail**
150%	103.1	85.8	17.3	**3.3**	**31.3**	**Fail**
Overall	102.6	99.3	3.3	**-15.1**	**21.9**	**Fail**

- Equivalency criteria were met between new RGA and the antigen-binding ELISA and TCR SPR assays.
- Equivalency criteria were **not** met between the new RGA and old RGA.
  - Triggered follow-up investigation to determine reason for non-equivalency per bridging study protocol.

Re-developed RGA versus Original RGA: Equivalency of Accuracy

Variability in the accuracy of all potency assays

Variability of reportable %RP of all potency assays

%RP Level	%CV			
	New RGA	Old RGA	ELISA	SPR
50	9.1	5.7	5.2	1.6
100	9.2	65.5	3.9	2.3
150	7.6	19.1	2.9	2.2

Conclusion: Accept non-equivalencies

1. Non-equivalencies due to improved precision of the re-developed RGA
2. Justifies why assay was re-developed

Re-developed RGA versus Binding Assays: Equivalency of DS and DP Potency Results

Equivalency in potency lot-release results between new RGA and other assays

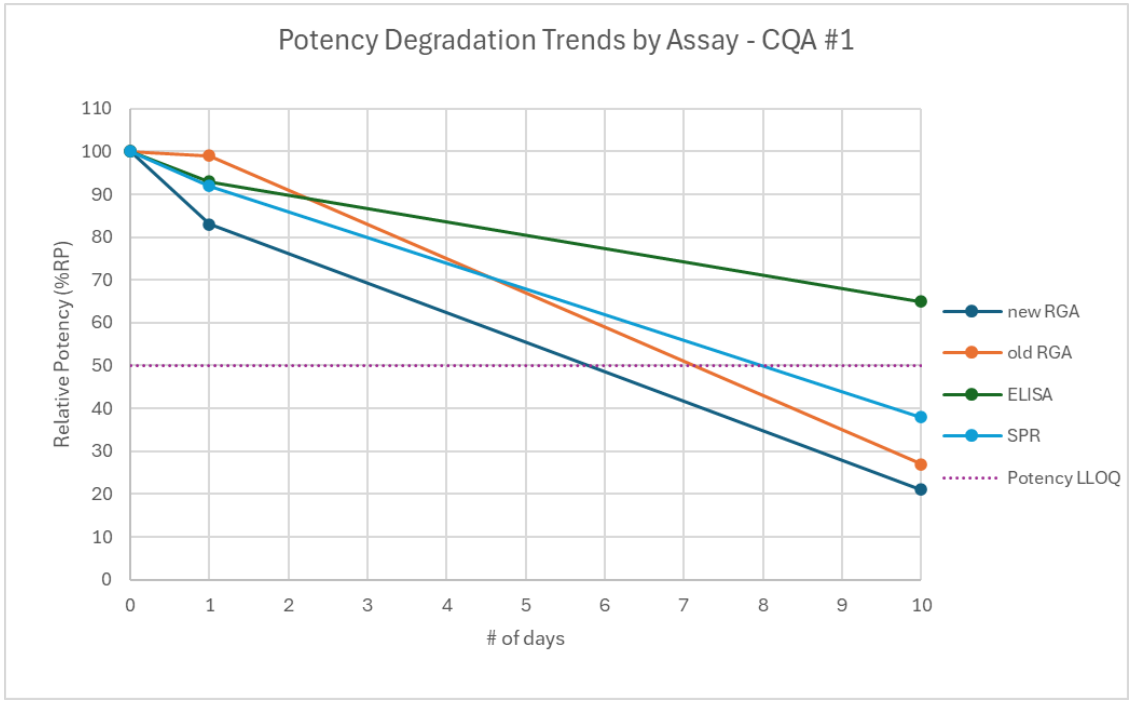
Lot	Reportable Result (%RP)			
	New RGA	Old RGA	ELISA	SPR
Lot #1	109	104	100	104
Lot #2	101	116	108	111
Lot #3	103	111	99	102
Lot #4	103	106	110	107
Lot #5	107	99	112	109
Lot #6	94	101	104	111

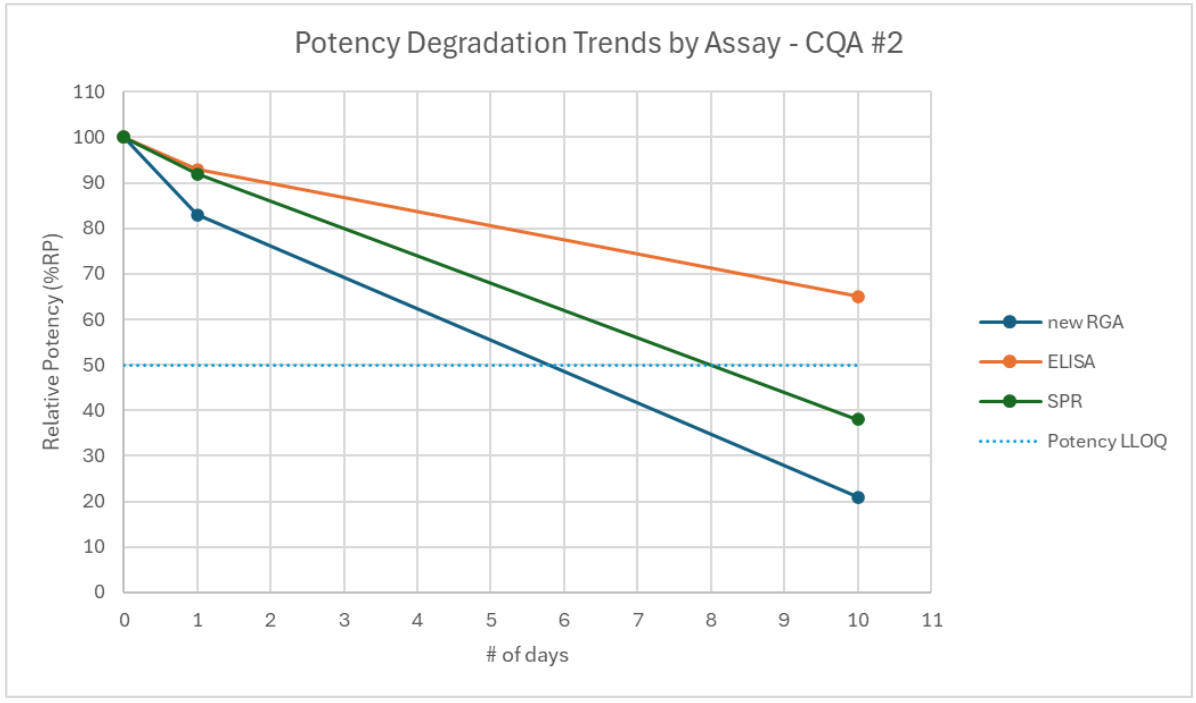
Conclusion: Re-developed RGA generates DS and DP lot-release testing results that are equivalent to all other methods

Confidence intervals between different method's testing results

Comparison	Mean Difference	90% LCL	90% UCL	Outcome
New RGA vs old RGA	-3.3	-10.4	3.7	Pass
New RGA vs ELISA	-2.7	-8.8	3.5	Pass
New RGA vs SPR	-4.5	-11.0	2.0	Pass

Re-developed RGA versus other Assays: Equivalency of Stability-Indicating Properties





Note: CQA #2 was discovered after bridging study completion, so these samples were not tested in the old RGA as that method had already been replaced with the new RGA.

- **Results show that new RGA is stability-indicating and sensitive to degradation at both Fab regions.**

Summary

Summary: Execution and analysis of bridge successful

1. Re-developed RGA, Antigen-binding ELISA, and TCR SPR assays demonstrate equivalent performance
2. Re-developed RGA shows decreased variability compared to the original RGA
3. RGA is cell-based and fully-MoA reflective assay

Overall: Re-developed RGA is suitable as a stand-alone lot-release potency method

Next Steps

1. **Immediately replaced original RGA with re-developed RGA for all testing**
2. **Implemented new potency strategy for GMP release and stability testing at time of implementing new manufacturing process.**
   - Antigen-binding ELISA and TCR SPR to be used for characterization only (ie process comparability studies)

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